Astragaloside IV improves cognitive impairment caused by CCH via improve ROS and NLRP3 pathway by up-regulating the PGC1α/Nrf2 pathway

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Abstract

This study was conducted to elucidate the possible molecular mechanisms of neuroprotective effect of Astragaloside IV (As-IV) on animal models of Vascular Dementia . Oxidative damage and neuroinflammation play a key role in chronic cerebral hypoperfusion (CCH) and lead to a decline in cognitive function in animals. In the present study, Sprague Dawley (SD) rats were randomly divided into sham group, model group, As-IV_{20} group, and As-IV_{50} group, with 10 rats in each group. The Morris water maze (MWM) was used to evaluate the cognitive function of rats. The nitrogen-blue tetrazolium photoreduction method was employed to detect the levels of superoxide dismutase (SOD) in the hippocampal tissue homogenate. The bituric acid method was utilised to detect the malondialdehyde (MDA) levels. Western blotting was performed to evaluate the levels of stimulator of interferon genes (Sting), NOD-like receptor protein 3 (NLRP3) Caspase-1 and Peroxisome proliferator-activated receptor gamma co-activator-1α (PGC-1α) and transcription factors such as nuclear factor erythroid-derived factor-2 (Nrf2) in hippocampus tissues. The results indicate that As-IV supplementation significantly regulates the above changes. In addition, As-IV treatment effectively up-regulates the PGC-1α and Nrf2. Overall, our findings suggest that As-IV can effectively improve the cognitive impairment caused by CCH and oxidative damage. Furthermore, mitochondrial damage and inflammation against CCH may be attributed to the activation of the PGC-1α/Nrf2 signalling pathway to reduce ROS. This activation can reduce neuroinflammation caused by Sting/NLRP3/caspase1 pathway.

1. Introduction

Vascular pathological changes and atherosclerosis previous stroke events are important risk factors for VaD, one of the most common forms of dementia, as a stage of cognitive impairment (1, 2). The clinical manifestations are cognitive decline and the decline in the ability to live independently, which are also common complications of stroke. Vascular dementia, the second most common type of dementia after Alzheimer's disease, significantly impairs the quality of life of patients and places a heavy burden on the health care system. CCH is considered to be an ideal model for VaD(3). CCH can cause a series of pathophysiological events, including inflammation and oxidative stress(4). Therefore, molecules or genes which could selectively suppress these pathophysiological events would be of great therapeutic interest in the treatment of VaD.

The latest research confirms that oxidative stress signal mediates inflammation activation(5). Furthermore, oxidative stress damage plays an important role in the pathogenesis of VaD(6). Oxidative stress refers to the excessive production of highly reactive oxygen species and highly reactive nitrogen species in the body after the organism is subjected to various harmful stimuli, which exceeds the ability of the body to remove oxides(7). This usually leads to tissue damage. Commonly used indicators of oxidative stress include superoxide dismutase (SOD) and malondialdehyde (MDA)(8). The past study found that the activity of SOD in the hippocampus of 2VO rats was significantly lower, and the level of MDA was higher than that of normal rats (9). Thus, enhancing the activity of antioxidant enzymes in the

body, scavenging oxygen free radicals, and inhibiting lipid peroxidation are the main methods to reduce the occurrence and development of VaD.

Previous studies have shown that the activation of the PGC-1α/Nrf2 pathway enhances the activity of SOD and eliminates active oxygen coming from different sources(10, 11). PGC-1α is a member of the PGC-1 family and a nuclear transcription coactivator. It works with PPARγ to enhance gene transcription activity, regulates the expression of mitochondrial biosynthesis-related genes to eliminate the generation of ROS, serves a role in cell protection, and maintains metabolic homeostasis(12). PGC-1α plays an important role in regulating brain energy metabolism and is associated with a variety of neurodegenerative diseases(13, 14). It has been reported that PGC-1α also activates transcription factors such as Nrf1 and Nrf2 for the advertisers of mitochondrial translation factor A (Tfam) to prompt mitochondria function, maintenance, and biogenesis(15). Nrf2 is a redox-sensitive transcription factor that provides cellular protection against oxidative damage triggered by neuronal dysfunction, injury, and inflammation. Under oxidative stress, Nrf2 dissociates from its inhibitor (Kelch-like ECH-associated protein 1, KEAP1). Subsequently, Nrf2 translocates to the nucleus and forms Nrf2/small MAF heterodimer, which binds to specific antioxidant response elements (16), and then activates the expression of a series of many antioxidant genes, including SOD, hydrogen peroxide Enzyme (CAT), glutathione s-transferase (GST). This removes a variety of oxidative substances from the body, improving neuroinflammation and neuronal apoptosis(17).

The exhaustion of endogenous antioxidants and the loss of mitochondrial function usually leads to neuronal death through programmed cell death. This results in changes in behaviour and histology. All of these mechanisms are accompanied by the rupture of the BBB, leading to oedema. Oedema can activate immune cells to secrete pro-inflammatory cytokines. Therefore, oxidative stress, mitochondrial function, and inflammatory factor pathways are closely interrelated and affect each other.

Neuroinflammation significantly affects cognition(18, 19). Both Sting and NLRP3 regulates inflammation and are involved in the innate immune response in multiple diseases(20-22). Innate immunity has a wide range of functions and is not immune to specific antigens, so it is also called non-specific immunity. It is the first line of defence against the occurrence and development of neuroinflammation and nervous system diseases. It can be triggered by damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Pattern recognition receptors of the innate immune system include Toll-like receptors (TLR), Nod-like receptors (NLR), and cGAS-Sting signalling pathway. The NLRP3 belongs to the subfamily of NLR. It activates caspase-1, thus causing the production of pro-inflammatory cytokines (IL-1β and IL-18) and pyroptosis(23). On the other hand, the Sting belongs to the cGAS-Sting signalling pathway. When the body is negatively stimulated or suffers an infection by pathogens, DNA gets damaged and leaks into the cytoplasm. Cytoplasmic cyclic GMP-AMP synthase (cGAS) detects the damaged DNA and produces cyclic guanosine monophosphate adenosine monophosphate (cGAMP), which binds to Sting as a second messenger. Sting subsequently co-localises with TBK1 and IRF3, leading to the induction of IFN-I. NLRP3 participates in immune regulation as a

downstream of Sting. However, the precise regulatory mechanisms of NLRP3 and Sting in VaD remain unclear.

As-IV is the main active component in the medicinal plant Astragalus membranaceus, which has been widely used in traditional Chinese medicine for the treatment of many diseases, including ischemic cerebrovascular disease, liver disease, kidney disease, and cancer(24, 25) . Many studies have shown that As-IV exhibits many pharmacological effects, such as anti-inflammatory, antioxidant, and anti-apoptosis. Thus, this study investigated whether As-IV could alleviate CCH-induced dementia via the PGC-1α/Nrf2 pathway. It also explored whether As-IV could improve oxidative stress and then influence the inflammatory factors, such as the Sting, NLRP3, and inflammasome.

2. Methods

2.1 Animal Preparation

All healthy and clean Sprague Dawley rats were between 7 and 8 weeks old, weighing 180±20g, and were purchased from Vital River, Beijing, China. All animal care and experimental procedures were approved by the animal care and management committee of Hebei General Hospital (permit number SCXK20160006). All data obtained were reported in compliance with ARRIVE guidelines. The rats were raised for 1 week before the experiment in the animal laboratory of Hebei General Hospital at a constant environment of 24 ± 2 °C with an alternating 12 h light-dark cycle. All rats were allowed free access to water and food.

All rats were divided into four groups randomly as follows: Sham group: rats received the sham operation (S, n=12); 2VO group: rats received the 2VO surgery (2VO, n=10); As- IV20 group: 20 mg/kg of As-IV treatment and 2VO surgery (As- IV20, n=10); As- IV50 group: 50 mg/kg of As-IV treatment and 2VO surgery (As- IV50, n=10).

2.2 Vascular Dementia Model

The 2VO group, As-IV20 group, and As-IV50 group underwent 2VO surgery, whereas the sham group was given sham surgery. First, animals were anaesthetised by intraperitoneal injection of 10% chloralhydrate (0.3ml/100g; Yongda Chemical Reagent Co., Ltd., Tianjin, China). Then, the bilateral common carotid arteries were exposed and separated from the attached tissues at 37.0 ± 0.5°C using a heating lamp (10). Subsequently, the bilateral common carotid arteries were double-ligated with 4-0 silk sutures and cut between the ligations in the 2VO group, As-IV20 group, and As-IV50 group. However, they were not ligated in the sham group. Then, every two animals were placed in a cage with free access to food and water for 4 weeks. After the surgery, the As-IV20 group and As-IV50 group were given the As-IV for 4 weeks.

2.3 Morris Water Maze Test

An MWM test was performed after 4 weeks of the surgery to assess for spatial learning and memory ability. The MWM (Shanghai Jiliang Software Technology Co., Ltd., Shanghai, China) was carried out in a black and circular pool (160 cm in diameter and 45 cm in depth) that was partially filled with water (at a

temperature of 23 ± 1°C). The maze was divided into four equal quadrants by four poles along the perimeter of the pool. The maze was located in a dimly lit, quiet test room with visual cues, which were maintained at fixed positions and could be used by rats for spatial orientation. During the training period, a black platform was placed in the centre of the same quadrant and submerged 2 cm below the surface of the water. A digital camera system was used to automatically track swimming trajectories and transfer parameters to an electronic image analyser. All experimental animals were trained 4 times for 5 consecutive days. In each trial, a rat was placed into the water facing the pool wall at one starting position. The time a rat took to reach the platform (escape latency) was recorded in each test. In this trial, the escape latency was recorded as 120s. If the rats failed to locate the hidden platform within 120s, they were gently guided to the platform where they were permitted to remain for a 10-second interval, and an escape latency of 120 seconds was assigned. On the sixth day, a spatial probe test was performed. The platform was removed, and the rats were released from the quadrant, which was opposite to the target quadrant and allowed to swim freely for 120s. The amount of time animals spent in the target quadrant (where the platform was previously located) and across the platform was recorded.

2.4 Superoxide Dismutase (SOD) and Malondialdehyde (MDA) Activity Assay

The tissue levels of antioxidant indices, including SOD and MDA, were measured using commercial kits according to the instructions of manufacturers.

2.5 Immunohistochemical Staining (IHC)

After the MWM test, paraffin-embedded sections were used to assess the expression of NLRP3 and Sting, according to standard histological procedures (n= 3 per group). Brain tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; 0.01 M, pH 7.4) over 24 h at 4 °C and then dehydrated in a graded series of alcohols and embedded in paraffin. Brain tissues were cut at 5 μm using a Leica® RM1850 rotary microtome (Leica Microsystem, IL, Hesja, Germany). Brain sections were incubated in 3% H2O2 to eliminate the endogenous peroxidase activity and 3% normal goat serum, then incubated with rabbit polyclonal antibody of NLRP3 (1:50 Proteintech), and Sting (1:50, Proteintech) in 0.01 M·L−1PBS overnight at 4 °C. Subsequently, they were rinsed with PBS and incubated with secondary antibodies at 37 °C for 45 min. Then they were rinsed again with PBS and incubated with secondary biotinylated conjugates at 37 °C. Slices were developed with diaminobenzidine and counterstained with haematoxylin. The secondary antibodies, secondary biotinylated conjugates, and diaminobenzidine from the streptavidin-peroxidase kit (Zhongshan Biology Technology Company, Beijing, China) were used to visualise the signals.

2.6 Immunofluorescence Staining

The other mounted slides from the same site were used in the immunofluorescence staining. The coronal sections handled by a sledge microtome were dewaxed, hydrated, and blocked with 10% normal goat serum for 1 h at 37°C. Then, the sections were incubated with rabbit anti-caspase1 (1:50, Proteintech) and mouse anti-GFAP (1:50, Proteintech) overnight at 4 °C. Subsequently, the sections were thoroughly

washed with PBS three times for 10 min each. Then, sections were incubated with anti-rabbit tetramethylrhodamine-conjugated secondary antibody (1:100) and anti-mouse FITC-conjugated secondary antibody for 50 mins at room temperature. Following that, sections were washed thoroughly with distilled water, dehydrated, cleared, and mounted on slides using anti-fading Fluoro-mount aqueous mounting media (Sigma). Then, a microscopic examination was conducted using a fluorescence microscope (Olympus, Japan) with the appropriate excitation/emission filters.

2.7 Western Blot Analysis

Following the MWM test, four rats from each group were randomly chosen to be decapitated under deep anaesthesia, and their bilateral hippocampi were dissected on the ice board. Proteins were extracted from the hippocampus using a total protein extraction kit (Solarbio, Beijing, China) following the manufacturer's protocols. The protein concentration of the supernatant was examined using a bicinchoninic acid protein assay reagent kit (Solarbio, Beijing, China) with bovine serum albumin as the standard. An equivalent amount of 50 μg total protein samples were separated to electrophoresis on 10-12% sodium dodecyl sulfate-polyacrylamide gels and transferred onto PVDF membranes (Millipore, Beijing, China). Then, the membranes were blocked for 1 h at 37 °C with bovine serum albumin in 0.1% Tween-20 in Tris-buffered saline (TTBS) then incubated with the following corresponding antibodies: (NLRP3, 1:1000 Proteintech; Caspase 1, PGC-1α, Nrf2) at 4°C overnight. GAPDH (1:50000 Proteintech). The next day, membranes were washed with TBST 3 times for 10 mins and subsequently incubated in TBST containing second antibodies (1:10000 dilution) for 1h at room temperature. Then, membranes were washed three times with TBST for 10 mins. Proteins bands were measured by an ECL detection kit and exposed to Amersham Imager 600. Finally, the optical density of all bands was quantified by AlphaEaseFc software.

2.8 Statistical Analyses

Except for neurological and behavioural scores, the data were analysed by a one-way analysis of variance (ANOVA), followed by Student–Newman-Keuls (SNK) test. The non-parametric ANOVA (Kruskal–Wallis one-way ANOVA on ranks), followed by Mann–Whitney test, was used to compare neurological scores. All values were expressed as the mean±SEM. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1 Astragaloside IV Can Improve Cognitive Impairment Caused by CCH

The escape latency in the 2VO group was significantly longer than the sham group from day1 to day5. This suggests that the VaD model was successful.Compared with sham group,the As- 50 group had a shortened escape latency on the first day ($P<0.05$).There was no significant difference from day 2 to day 5. There was no statistically significant difference in escape latency between sham group and As-20. Compared with 2VO group, the escape incubation period of As- 50 group had a shortened escape

latency within 5 days (P <0.01); As- 20 group also had a shortened escape latency (Day1 P <0.01 Day 2 P <0.05; Day 3 P <0.01; Day 4: P <0.05). Compared with As- 50 group, the escape latency of As- 20 group was different on the first, third and fourth days, and As- 50 group had good results (P <0.05). The results of spatial probe test revealed that 2VO group spent less time in the target quadrant than those in Sham group (P <0.01). As- 50 group and As- 20 group spent longer time in the target quadrant than the 2VO group (P <0.01).

The MWM test data of each group are shown in Figure 1.

3.2 As-IV Can Reduce CCH-Induced ROS Damage

We used the commercial kits to evaluate the levels of SOD and MDA. Compared with the sham group, a significant decrease in SOD and increase in MDA were observed after 2VO surgery (level of SOD: P <0.01; level of MDA: P <0.01;). However, the intervention of As-IV reversed these changes (level of SOD: P <0.01, As-IV_{20} vs 2VO group, P <0.01, As-IV_{50} vs 2VO group; level of MDA: P <0.01, As-IV_{20} vs 2VO group, P <0.01, As-$IV_5$0 vs 2VO group). The treatment effect of the As-IV_{50} group was better than the As-IV_{20} group (level of SOD: P <0.01; level of MDA: P <0.01).

The level of SOD and MDA test data of each group are shown in Figure 2.

3.3 As-IV Can Activate the PGC-1α/Nrf2 Signalling Pathway

We used western blotting to detect the protein levels of PGC-1α/ Nrf2 to investigate whether the PGC-1α/ Nrf2 pathway was involved in the good effect of the As-IV. After 2VO surgery, the levels of these factors were significantly reduced compared with the sham group (protein level of PGC-1α P <0.001; protein level of Nrf2: P <0.001;). However, after treatment with As-IV, the levels of these factors were restored (protein level of PGC-1α: P <0.001, As-IV_{20} vs 2VO group, P <0.001, As-IV 50 vs 2VO group; protein level of Nrf2: P <0.001, As-IV20 vs 2VO group, P <0.001, As-IV50 vs 2VO group). The protein levels of PGC-1α, Nrf2 test data of each group are shown in Figure 3.

3.4 As-IV Can Reduce CCH-Induced Neuroinflammatory Response through Sting/NLRP3/caspase1 Pathway

In order to investigate whether As-IV could improve cognitive behaviour by inhibiting inflammatory pathways, we carried out a western blot assay to detect Sting, NLRP3, caspase1, and GFAP. Subsequently, an immunofluorescence double-labelling was performed to detect caspase1 and GFAP Neun. After 2VO surgery, the expression of these inflammatory factors in the model group was significantly higher than that in the sham operation group (protein level of Sting: P <0.001; protein level of NLRP3: P <0.01; protein level of caspase1: P <0.01; protein level of GFAP: P <0.01). However, the application of As-IV treatment significantly alleviated this inflammatory response. (protein level of Sting: P <0.01, As-IV_{20} vs 2VO group, P <0.01, As-IV_{50} vs 2VO group; protein level of NLRP3: P <0.01, As-IV_{20} vs 2VO, P <0.01, As-IV_{50} vs 2VO

group; protein level of caspase1: P <0.01, As-IV_{20} vs 2VO group, P <0.01, As-IV_{50} vs 2VO group; protein level of GFAP: P <0.01, As-IV_{20} vs 2VO group, P <0.01, As-IV_{50} vs 2VO group).

We examined the expressions of caspase1 in neurons and astrocytes. In the sham group, caspase1 was detected in fewer neurons and almost no astrocytes. In the 2VO group, the expression of caspase1 was mainly detected in the neurons, few in the astrocytes. Compared to the sham group, the 2VO group showed a significant increase in the number of GFAP-positive cells and upregulation of GFAP expression. GFAP immunoreactivity was observed in the area between and tightly surrounded pyramidal neurons.

The protein levels of Sting, NLRP3, caspase1 and GFAP test data of each group is shown in Figure4.

The Immunohistochemical staining of NLRP3 is displayed in Figure 5.

The double Immunofluorescence staining of caspase1, Neun, or/and GFAP are exhibited in Figure 6.

4. Discussion

In the current study, we examined the neuromodulatory effect of As-IV in a VaD model of CCH through an evaluation of common pathological features such as inflammation, oxidative damage, mitochondrial biogenesis, neuronal loss, and degeneration. Oxidative damage and inflammation are known to be hallmarks of VaD pathology. They have been extensively studied for their possible role in neurodegeneration after chronic injury. The present study found that As-IV led to a significant improvement of cognition, reduced the oxidative damage and inflammatory response in CCH rats. As-IV supplementation immediately following CCH was also revealed to activate the PGC-1α/Nrf2pathway. To our knowledge, this was the first study that evaluated the modulation of the PGC-1α/Nrf2 signalling pathway by As-IV in a CCH model of VaD. The findings indicate that As-IV improves cognitive impairment caused by CCH by up-regulating the PGC-1α/Nrf2 pathway, consequently improving ROS and Sting/NLRP3/caspase1 pathway.

Vascular risk factors, including hypertension, diabetes, hyperlipidemia, smoking, atrial fibrillation, and hyperhomocysteinemia, are found to play a role in the development of dementia. Currently, there is accumulating evidence that the damage of neurovascular unit (NVU) is the main cause of vascular cognitive dysfunction(26). The NVU consists of neurons, astrocytes, microglia, interneurons, pericytes, vascular smooth muscle cells (VSMCs), and endothelial cells(27). There are many potential mechanisms for vascular risk factors to lead to vascular damage. For example, oxidative stress causes vascular endothelial dysfunction, reduced cerebral vascular flow, the destruction of the blood-brain barrier, and tissue hypoxia. It can further pass through hypoxia, cause inflammatory changes and vascular cells, reactivate astrocytes, and activate microglia to produce cytokines. These mechanisms can lead to nutrient uncoupling of neurovascular units and damage myelin sheath. Once demyelination occurs, the energy demand of the exfoliated axons increases, which aggravates the hypoxic stress of the tissue. This results in a vicious cycle, continuing these pathogenic processes and aggravating tissue damage. Thus, oxidative stress and inflammation play a key role in the pathogenesis of vascular cognitive impairment.

Therefore, investigating potential drugs and methods that can reduce the occurrence and development of oxidative stress and inflammation during the pathogenesis is crucial for the disease to be controlled.

We found that CCH induced behavioural impairments as evidenced by performance in MWM. In both the positioning cruise experiment and the space exploration experiment, the 2VO group showed a lower ability to complete the task than the sham group. In addition, the ability of the two As-IV groups after treatment was better than that of the 2VO group. These findings indicate that after As-IV treatment, the cognitive ability of CCH rats has recovered, and the treatment effect of the high-dose group was better than that of the low-dose treatment group. Astragaloside IV is the most effective component of traditional Chinese medicine, Astragalus membranaceus. Several studies have shown that it plays a protective effect in tumour diseases, acute and chronic kidney damage, and cardiovascular and cerebrovascular diseases, by regulating oxidative stress, improving immunity, and regulating inflammation pathways(28-30).

Oxidative stress is associated with ageing. It is considered to be the root cause of most diseases and body ageing. Under normal circumstances, the defence system of the body effectively resists oxidation and generates free radicals in the body. This system protects cells from damage. However, when the body is subjected to various harmful stimuli, it produces various highly active molecules, including reactive oxygen species (ROS) and reactive nitrogen species(1) . When the oxidation degree of excessive free radicals far exceeds the scavenging ability of the body, cells get damaged(31). In other words, when the body is subjected to various harmful stimuli, the oxidation and antioxidant systems in the body and cells get out of balance, leading to infiltration of inflammatory cells and increased secretion of related proteases, resulting in a large number of oxidation-related intermediate metabolites. Previous studies have found that oxidation plays a key role in the ischemic injury of VD neurons. MDA and SOD are commonly used oxidative stress indicators. MDA is the final product of the oxidation reaction that gets generated between ROS and lipid components of biological membranes and is positively correlated with the degree of oxidative stress and lipid peroxidation. It can directly reflect the degree of lipid peroxidation of cells and indirectly reflect the severity of the cells being attacked by free radicals. On the other hand, SOD is the main redox regulating enzyme in tissue cells, which can catalyse the scavenging reaction of superoxide free radicals in organisms. It can scavenge oxygen free radicals, effectively reduce the excessive ROS generation after tissue damage, and accelerate the scavenging of free radicals, thereby protecting tissue cells. Its activity reflects the functional state of oxidative stress. When the body tissue is damaged by oxidative stress, the homeostasis is destroyed, the SOD level is reduced, and the MDA level is promoted to increase. These mechanisms cause neuronal damage and promote a vicious circle of oxidative stress damage in brain tissue. The activity of SOD and MDA enzymes were measured spectrophotometrically to investigate the level of oxidative damage after CCH. The findings demonstrated that the increased MDA and reduced SOD levels in the rat hippocampus were indicators for CCH induced oxidative damage. As-IV was found to improve this damage from oxidative stress. ROS regulation of signal transduction allows cellular pathways to rapidly adapt to changes in the oxidative environment. Shaw indicated that SAH induced an increase in the MDA level, neuronal apoptosis, cleaved caspase 3, brain enema and decreased activities of SOD and glutathione peroxidase (GSH-Px). However, As-IV

treatment reversed these changes and improved neurobehavioral outcomes of SAH rats(32). Yang demonstrated that As-IV protects dopaminergic neurons from neuroinflammation and oxidative stress(33). Similarly, Chen noted that As-IV protects the renal tubular epithelial cells from free fatty acids-induced injury by reducing oxidative stress(34). Furthermore, As-IV significantly enhances PKA and CREB phosphorylation and prevents OGD-induced mitochondrial dysfunction, thereby protecting neurons exposed to OGD from injury and death(35).

ROS is a by-product of mitochondrial oxidative phosphorylation. Mitochondrial dysfunction can cause excessive ROS production. PGC-1α is a key protein in mitochondrial biosynthesis. It plays an important role in regulating mitochondrial function and also coordinates the expression of multiple antioxidant genes, protecting the body from oxidative stress damage. In neuronal cells, PGC-1α can up-regulate the expression of antioxidant genes such as SOD, SOD2. In recent studies on ageing models, it has been found that PGC-1α regulates the expression of Nrf2 and thus jointly regulates the antioxidant effect. Nrf2 is also an important molecule that modulates oxidative stress and plays an important role in cerebrovascular diseases and neuroinflammatory pathways. The promoter region of PGC-1α contains the same sequence as Nrf2. Thus, it is speculated that there may be a redox regulatory loop between PGC-1α and Nrf2. Furthermore, PGC-1α and Nrf2 may be regulated by the same signal pathway. Peroxisome Proliferator Receptor Activated Receptor γ (PPARγ) is the main component that maintains mitochondrial function and oxidative metabolism. Under stress conditions, PPARγ is transcriptionally activated by PGC-1α and then binds to the promoter region of Nrf2 to activate its transcriptional activity(36). Through this mechanism, PGC-1α-mediated Nrf2 expression not only leads to the enhancement of antioxidant capacity but also induces the expression of mitochondrial genes through a new signal axis. In summary, Nrf2 and PGC-1α play a synergistic role in maintaining redox balance and mitochondrial function homeostasis. Our findings indicate that the PGC-1α and Nrf2 of the hippocampus in the 2VO group were significantly lower than those of the sham group. After As-IV treatment, these indicators were increased compared with the 2VO group. However, there was no significant difference between As-IV_{20} and As-IV_{50} groups. This is consistent with the overall trend of the NLRP3 pathway, indicating that As-IV can regulate oxidative stress and inhibit neuroinflammation by up-regulating the PGC-1α/Nrf2 pathway and play a role in improving VaD. The activation of the melanocortin 1 receptor with BMS-470539 significantly attenuated early brain injury after SAH by suppressing the oxidative stress, apoptosis, and mitochondrial fission through the AMPK/SIRT1/PGC-1α signalling pathway(37). Zhang found that Platycodin D protected BV-2 cells from Aβ-induced oxidative stress and inflammation via regulating the TLR4/NF-κB and Nrf2/HO-1 signalling pathways. T-006, a new derivative of tetramethylpyrazine, stimulated MEF2, PGC-1α, and Nrf2 transcriptional activities, inducing Nrf2 nuclear localisation and playing a neuroprotective effect .

Neuroinflammation plays an important role in the occurrence and development of dementia. Although appropriate neuroinflammation has some neuroprotective effects, chronic and excessive neuroinflammation mostly exhibits neurotoxic effects(38) . GFAP, a biomarker for activated astrocytes, has recently been proposed as a new diagnostic biomarker for VaD(39). NLRP3 inflammasomes are widely present in immune and inflammatory cells. They are usually activated by external stimuli such as

microbes, stress, and damage signals. NLRP3 inflammasome can recognise pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). When stimulated, NLRP3 interacts with its adaptor ASC and activates caspase-1. Subsequently, activated caspase-1 can process pro-IL-1β and pro-IL-18 and transform them into their mature forms, ultimately amplifying the inflammatory response. Previous literature has confirmed that the NLRP3 inflammasome/caspase-l/IL-lβ signal axis is involved in the pathogenesis and progression of many diseases, making it a promising target for potential treatment. The production of ROS can activate the NLRP3 inflammasomes(40). The immunostimulatory and antiviral potential of DNA was discovered as early as 1963. Thus, it is known that the accumulation of foreign DNA or the own DNA of the cell in the cytoplasm causes a strong immune response. cGAS recognises cytoplasmic DNA and catalyses the synthesis of cGAMP by adenosine triphosphate (ATP) and guanosine triphosphate (GTP). Then, cGAMP activates Sting, which in turn activates TANK-binding kinase 1, TBK1, and inhibitor of nuclear factor kappa-B kinase. Subsequently, they activate interferon regulatory factor 3 (IRF3) and nuclear factor kappa-B kinase (NF-κB), which eventually causes a rapid and strong interferon production to resist pathogen invasion and tissue damage. Recent work indicates that ROS regulates cellular defence pathways, including Toll-like receptor signalling and inflammasome activation. It has also been reported that ROS inhibits Sting polymerisation and activation of downstream signalling events. Some studies suggested that Sting could promote NLRP3(41). Our results showed that the Sting and NLRP3 levels of the hippocampus in the 2VO group were significantly higher than that of the sham group. Moreover, after As-IV treatment, the above indicators were detected to be lower than that of the 2VO group; however, there was no difference between As-IV20 and As-IV50. This finding suggests that As-IV could alleviate cognitive deficit by inhibiting the Sting/NLRP3/caspase1 pathway. As-IV and cycloastragenol were found to suppress ROS-associated ER stress and then inhibit TXNIP/NLRP3 inflammasome activation with the regulation of AMPK activity, thereby ameliorating endothelial dysfunction by inhibiting inflammation and reducing cell apoptosis. Sun showed that As-IV could inhibit monocrotaline-induced pulmonary arterial hypertension via the NLRP3/calpain-1 pathway(42). Furthermore, Xie suggested that As-IV protects intestinal epithelium from sepsis-induced barrier dysfunction via inhibiting RhoA/NLRP3 inflammasome signal pathway(43). Moreover, As-IV lessened reactive oxygen species generation in LPS-induced BV2 microglia cells significantly. These findings demonstrate that As-IV protects dopaminergic neurons from neuroinflammation and oxidative stress, which are largely dependent upon activation of the Nrf2 pathways and suppression of the NFκB/NLRP3 inflammasome signalling pathway.

In the present study, the regulation trend of the Sting/NLRP3/caspase1 pathway and PGC-1α/Nrf2 pathway was opposite after the administration of astragaloside IV. However, neither was seen due to the improvement in the dose of As-IV, and the two pathways had a significant improvement. The results indicate that As-IV is closely involved in improving the mechanisms of dementia caused by CCH. However, since we did not use agonists or inhibitors, it is impossible to speculate which of the two acts on whom. Fortunately, many studies have relevant hints. For example, Liu showed that CPZ treatment significantly increases the expression of Trpv4, activates NLRP3 inflammasome, reduces peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), and decreases mitochondrial function.

Kai suggested that Oroxylin A inhibits NLRP3 inflammasome activation by reducing ROS accumulation and up-regulating the PGC-1α(44).

Our findings reveal that As-IV administration significantly attenuates oxidative damage and neuroinflammation after CCH via activation of the PGC-1α /Nrf2 pathway. A considerable body of literature shows that mitochondrial dysfunction, oxidative stress, and inflammation are unfavourable factors for many neurological diseases. Therefore, regulating the PGC-1α/Nrf2 pathway to regulate ROS generation and then the level of the Sting/NLRP3/caspase1 pathway may be a promising treatment strategy for the treatment of many neurological diseases.

5. Conclusion

The present study found that As-IV improves the degree of oxidative stress damage in a dose-dependent manner. However, no significant differences were observed between low dose and high dose in NLRP3 and PGC-1α. On the other hand, the results of the MWM suggest that there might be some other molecular mechanisms involved, such as autophagy, apoptosis, and endoplasmic reticulum stress. We will further analyse these indicators in our future research.

Declarations

Funding

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics approval

All animal care and experimental procedures were approved by the Animal Care and Management Committee of Hebei General Hospital (permit number SCXK20160006).

Consent to participate

Consent for publication

Not applicable.

Availability of data and material

The data used in this article are available to researchers subject to confidentiality if necessary.

Code availability

Authors' contributions

NM and PYL conceived and designed this work. NM performed the main experiments.JX,MXL, LZ, TYG, MJ analyzed the data and prepared the fig1-5. NM wrote the manuscript. ZJT,HYH,MYF prepared the fig.6. All authors read and approved the final manuscript.

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Figures

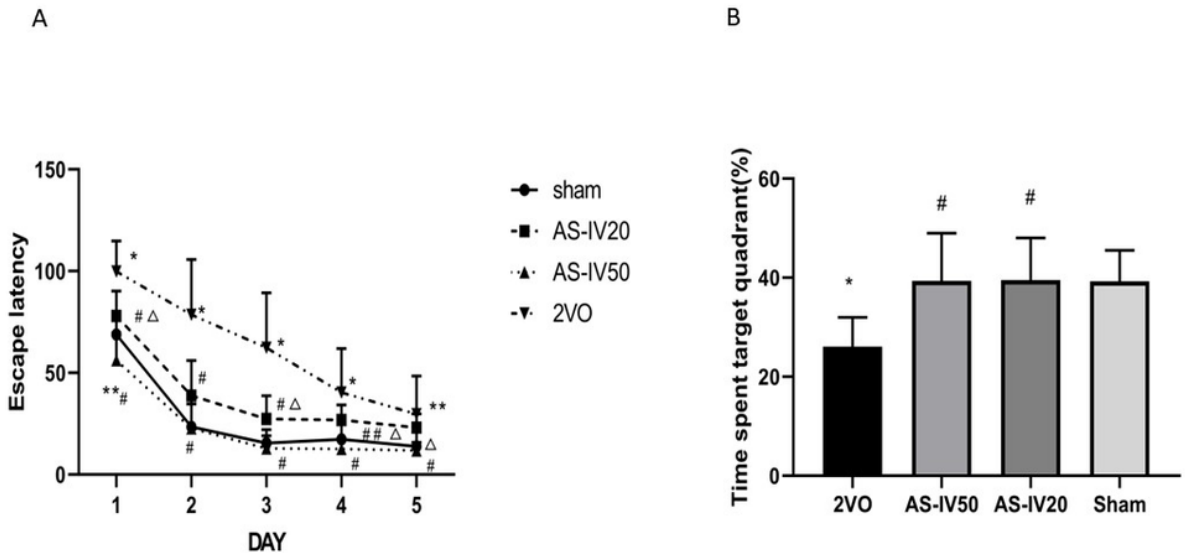

Figure 1

MWM

All animals underwent MWM behavioral assessment, and showed differences.*P<0.01 vs. sham group;**P<0.05 vs. sham group; #P<0.01 vs. 2VO group; ##P<0.05 vs. 2VO group △P<0.05 As- 20 group

vs. As- 50 group

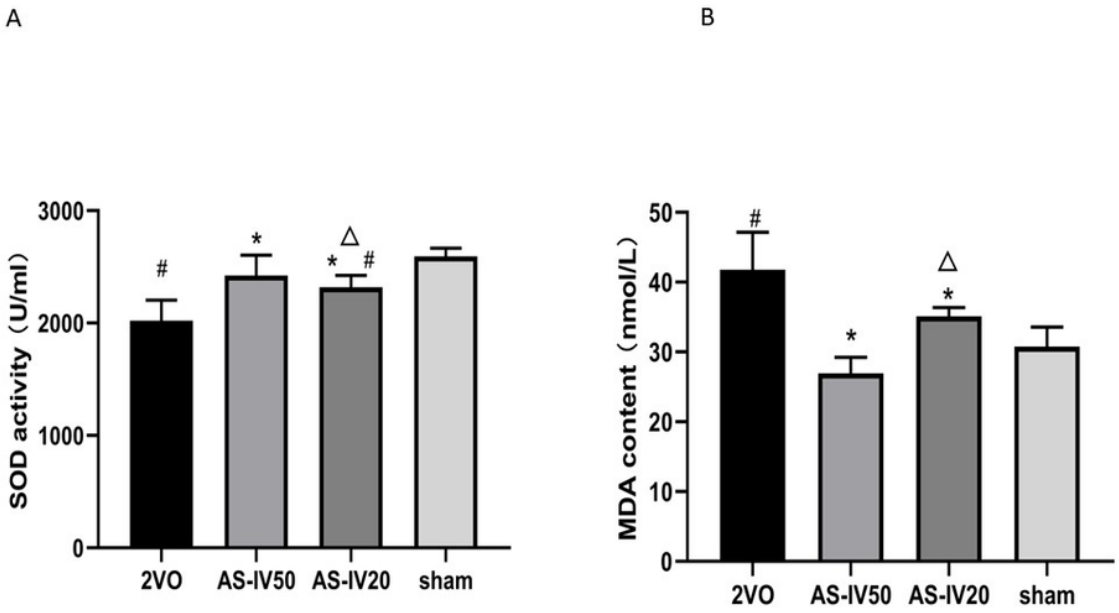

Figure 2

SOD MDA

The SOD MDA content of hippocampus in each group. *P<0.01 vs. 2VO group;#P<0.01 vs. Sham group; △P<0.01 As-IV20 vs.As-IV50 group.

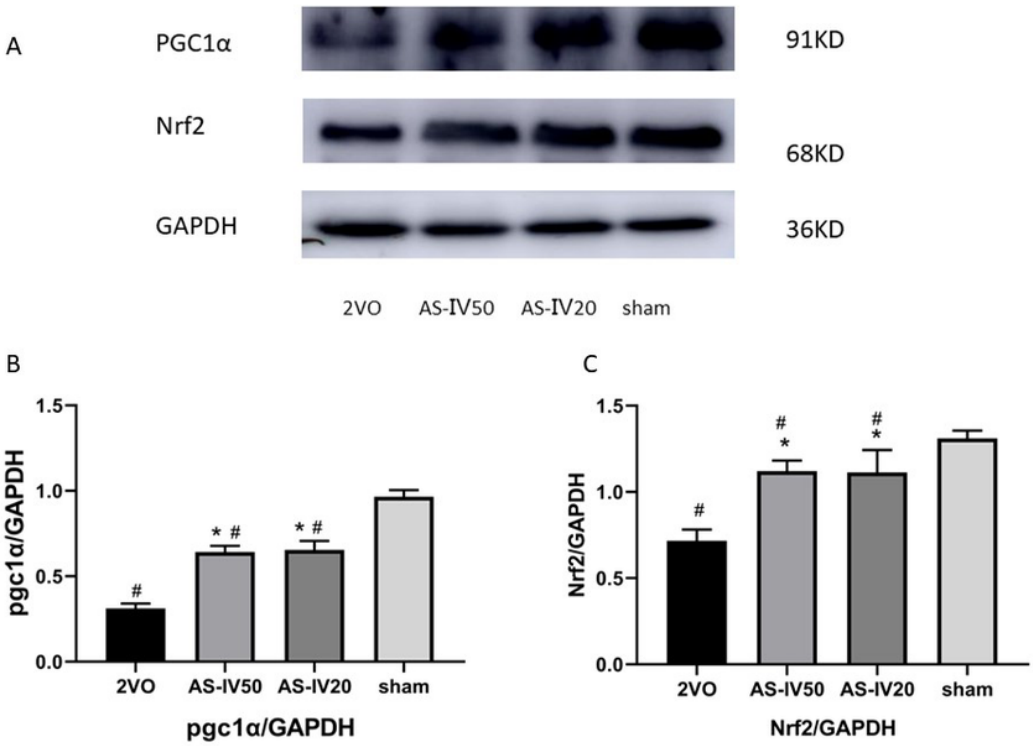

Figure 3

PGC1α/Nrf2 pathway

(A) Western blot analysis of the expressions of PGC1α and Nrf2 (n = 6 in each Group

(B) Quantitative analysis of protein levels of PGC1α.

(C) Quantitative analysis of protein levels of Nrf2.

#*P* <0.001 vs. Sham group;**P*<0.01 vs. 2VO group

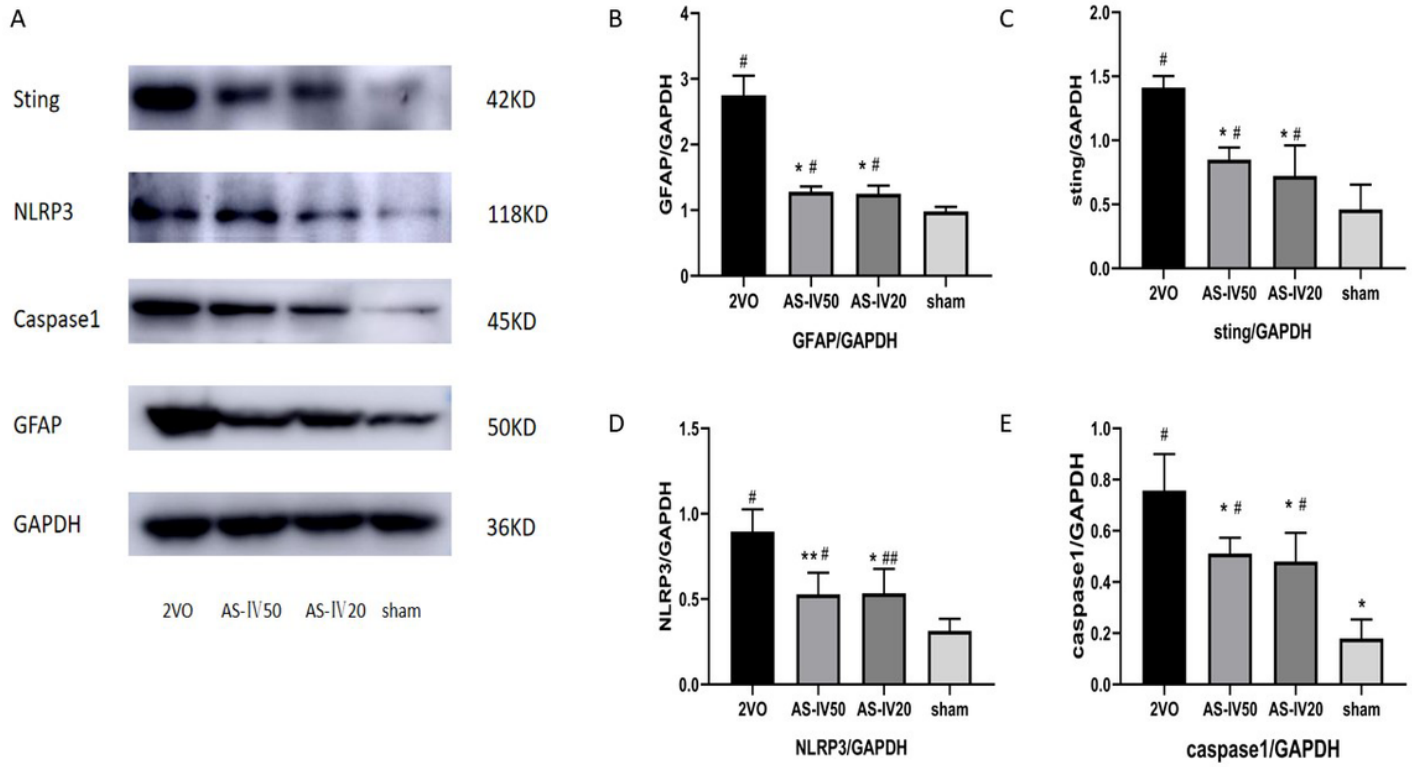

Figure 4

Sting/NLRP3/caspase1 pathway

(A)Western blot analysis of the expressions of **Sting,NLRP3,caspase1 and GFAP**

(n = 6 in each Group)

(B) Quantitative analysis of protein levels of GFAP

(C) Quantitative analysis of protein levels of Sting.

(D) Quantitative analysis of protein levels of NLRP3.

(E) Quantitative analysis of protein levels of caspase1.

#*P* <0.001 vs. Sham group ##*P* <0.01 vs. Sham group;

**P*<0.001 vs. 2VO group ***P*<0.01 vs. 2VO group

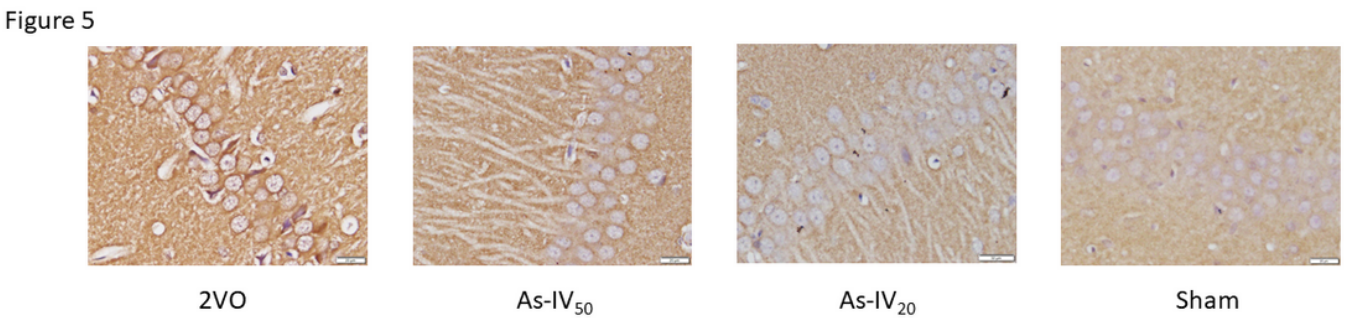

Figure 5

The Immunohistochemical staining of NLRP3

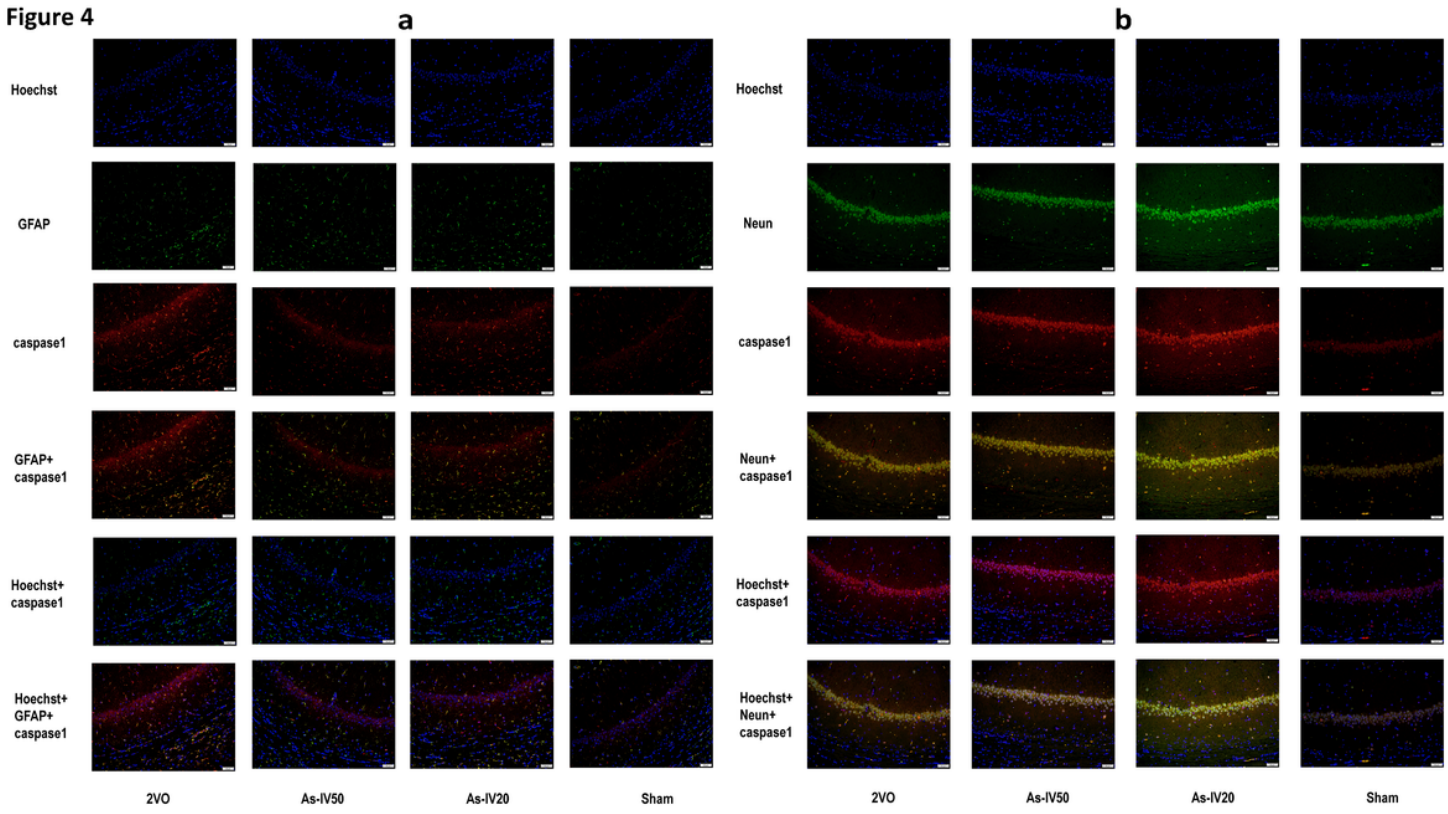

Figure 6

The double Immunofluorescence staining of caspase1, Neun, or/and GFAP

(a) Representative images of GFAP (red) and BrdU (green) immunofluorescence double labeling in corpus callosum at 2 weeks and 4 weeks after 2VO.